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Selection of leaf litter by Lumbricus terrestris

INTRODUCTION

Comprehension of the dynamics of soil communities rests at present on a rather primitive knowledge of the food, feeding behaviour and digestive metabolism of soil animals. One aspect which has however received considerable attention is the preferences shown by litter animals for different types of forest leaf litter presented to them in food selection experiments. Studies on Collembola, Acari, Isopoda, Diplopoda, Mollusca and Lumbricidae have shown broadly similar results in that litter of such species as elder, elm, alder and ash is generally preferred to that of oak, beech, pine and larch and that weathered litter is preferred to fresh litter. Agreement has not yet been reached on the relative importance in food selection of physical factors such as hardness and hairiness; water content; taste substances such as tannins and resins; and such chemical factors as the calcium content, protein content and C/N ratio.

This paper presents some interim results from a study of leaf litter selection by *Lumbricus terrestris* which, because of its habit of pulling surface litter into its burrows, is a convenient animal for selection experiments.

ESTABLISHMENT OF AN ORDER OF PREFERENCE FOR VARIOUS LITTERS

Experimental Methods

The litter selection experiments were carried out in a cellar approximately at soil temperature in garden soil contained either in large wooden boxes or in plastic dustbins. The factor of leaf shape was eliminated by

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using 1 cm diameter discs cut from the lamina of leaves of broad leaved species and 2 cm lengths of conifer needles. The discs or pieces of needle to be tested were placed on the soil in the containers and any which had not been pulled into worm burrows were removed and identified on the following day.

In the experiments carried out in the wooden boxes all the materials to be tested were placed on the soil in a grid pattern. The pattern was changed on succesive nights until all the litters under test had occupied all the positions in the grid. In the experiments in plastic bins, four test litters and a control sample of blotting paper discs were placed on the soil in each bin each night. The number of litter discs removed was recorded as a percentage of the controls removed. This allowed the tests made on different nights to be compared. In all these tests the leaf discs were kept for the previous 12 hours in a screw-topped phial containing just sufficient water to moisten them. The leaf litters were collected on sheets placed under the trees at leaf fall and stored fresh at -10°C. Weathered litter was obtained by placing some of the fresh litter outdoors in a thin layer between coarse-meshed nylon nets on a lawn. After the appropriate time it was stored at -10°C.

Results

Experiment 1, 1961 - The litters were collected from mull and mor sites and sub-samples were weathered as described for 6 weeks and 12 weeks. Six discs of each kind were given nightly for 20 nights.

Table 1: The proportion of leaf discs of various litters (%) taken by *Lumbricus terrestris* in a selection experiment

	Mor				Mull					
Weathering	Beech	Oak	Hazel	Birch	Beech	Oak	Hazel	Birch	Ash	Alder
	48	49	64	73	58	23	61	80	76	82
6 weeks	67	62	74	77	65	81	73	73	85	93
12 weeks	73	75	82	84	78	85	80	83	88	96

Experiment 2, 1962, included a number of pigmented litters and was carried out in the boxes with 20 discs of each kind given nightly for 12 nights.

Experiment 3, 1963, was carried out in the same manner with 14 discs or needle lengths of each kind given nightly for 21 nights.
Experiment 4, 1964, was carried out as before but with half the litter weathered for 6 weeks. Six discs or needle lengths were given nightly for 33 nights.
Experiment 5, 1965, was carried out in bins with blotting-paper controls, fresh litter and litter weathered for 24 days. Ten discs or needle lengths of each kind were tested in 30 bins. Thus, on the first night 300 discs of weathered birch, unweathered birch, weathered oak, unweathered oak and blotting-paper were tested. On subsequent nights sycamore was tested with beech, gean with pine, ash with hazel, lime with alder, spindle with larch and elm with lime. The results of all these experiments are given in Table 2.
These tests confirm the results of previous workers including those of Lindquist (1941) in showing that some litters are preferred by *Lumbricus terrestris* to others and that in most cases, weathered litter is preferred to unweathered litter of the same species. The order is not a precise one, varying to some extent in different tests using litters from different sources. Broadly speaking, litters of elder, elm, alder, black poplar, *Prunus serrulata,* ash and sycamore were highly palatable; birch, spindle, aspen, field maple, lime, guelder rose, *Prunus cerasifera,* hazel, horse chestnut, walnut and gean were moderately palatable; and Virginia creeper, beech, *Abies grandis,* pine, larch and oak were disliked.

PHYSICAL STRUCTURE

The preferences shown suggest at first sight some correspondence with the hardness, thickness, toughness or similar physical characteristic of the litter. A selection test was therefore carried out on litters in which cuticle and leaf thickness were measured from sections. The results (Table 3) show litters of similar thickness e.g. poplar and beech, with dissimilar palatability and with some thick species e.g. holly and plane, more palatable than thinner species.

The relative toughness of a range of litters was assessed by using a simple penetrometer. This comprised a stiff pointed wire held loosely in a glass tube and supporting a light plastic pan, the wire and pan weighing 5 g. The leaf under test was held between two polythene sheets with a central 1 cm diameter hole. The point of the penetrometer was

Table 2: Order of preference shown in litter selection experiments with *Lumbricus terrestris*

U = Unweathered W = Weathered	1962 U % removed	1962 U Order	1963 U % removed	1963 U Order	1964 U % removed	1964 U Order	1964 W % removed	1964 W Order	1965 U % removed	1965 U Order	1965 W % removed	1965 W Order
Elder Sambucus nigra	72	1	46	1	80	1	68	2				
Elm Ulmus glabra									64	1	63	2
Alder Alnus glutinosa			41	5	69	2	78	1	72	2	75	5
Black Poplar Populus nigra			43	4	68	3	66	5				
Flowering Cherry Prunus serrulata	64	2										
Ash Fraxinus excelsior	63	3	45	2	60	5	65	6	58	6	63	7
Sycamore Acer pseudoplatanus			45	2	68	4	68	3	43	3	55	1
Birch Betula verrucosa			26	6	60	5	67	4	45	4	55	3
Spindle Euonymus europaeus									50	5	62	4
Aspen Populus tremula					59	7	65	6				
Field Maple Acer campestre			26	6								
Lime Tilia europaea									58	7	71	6
Guelder Rose Viburnum opulus	54	4										
Prunus cerasifera var atropurpurea	51	5										
Hazel Corylus avellana			21	8	41	8	62	8	38	8	41	10
Horse Chestnut Aesculus hippocastanum			21	8								
Walnut Juglans regia			21	8								
Gean Prunus avium	42	6							37	9	60	8

Table 2 continued

U = Unweathered W = Weathered	1962 U % removed	1962 U Order	1963 U % removed	1963 U Order	1964 U % removed	1964 U Order	1964 W % removed	1964 W Order	1965 U % removed	1965 U Order	1965 W % removed	1965 W Order
Virginia Creeper Parthenocissus quinquefolia	11	7										
Beech Fagus sylvatica	9	8	18	11	17	11	30	10	12	10	26	9
Giant Fir Abies grandis			15	12	21	10	23	11				
Pine Pinus sylvestris			11	13	12	12	19	12	11	11	19	13
Larch Larix decidua					9	13	9	13	7	12	15	12
Oak Quercus petraea	4	9	8	14	38	9	36	9	3	13	28	11

Table 3: Leaf and cuticle thickness in relation to palatability

	Palatability % discs taken from 150	Mean thickness in μ Cuticle	 Whole leaf
Poplar	60	3	160
Holly	50	9	200
Plane	40	7	190
Oak	39	6	186
Beech	39	3	108

rested on the leaf lamina exposed in this central hole and balance weights were placed on the pan until the point punctured the leaf. Five punctures were made in each leaf in positions avoiding the midrib, secondary veins and fungal spots.

Measurements were made on air-dry leaves and on leaves which had been soaked in water for 24 hours. The values given in Table 4 are the means for each leaf. For pine, single readings were made on 25 needles. The

leaves and needles were taken from the litter used in Experiment 5 (1965). (Table 4 - see overleaf)

The values obtained, though somewhat crude, serve to show that there was little correspondence between leaf toughness and palatability. Thus sycamore, though more palatable than oak, was of much the same toughness.

In Experiment 5 the palatability of all litters increased during weathering but in birch, lime, hazel and pine the values obtained with the penetrometer were the same in weathered and unweathered litter. In the other litters the increased variability in mechanical strength with weathering suggests different degrees of attack by micro-organisms. The changes are, however, small by comparison with the initial variability of the samples and tend to confirm the view of Dunger (1958) that the mechanical nature of the litter is of rather small significance in its palatability.

Table 4: Weight (g) required for a penetrometer to puncture litters used in Experiment 5 (1965)

	Air-dry						Air-dry then soaked for 24 hours						Order of palatability in Expt. 5
Unweathered	Leaf	1	2	3	4	5 Mean	Leaf	1	2	3	4	5 Mean	
Elm		20	19	23	21	20 21		14	13	17	13	12 14	1
Alder		19	15	19	17	17 17		11	15	8	19	13 13	2
Sycamore		35	33	30	19	54 34		15	17	19	17	18 17	3
Birch		28	31	29	27	28 31		15	15	15	13	12 14	4
Lime		15	24	10	10	7 14		17	13	14	17	17 15	5
Hazel		14	14	22	17	27 19		11	16	11	12	11 12	6
Gean		30	31	28	28	30 30		23	21	21	22	20 22	7
Pine 25 needles				150-300		213				100-190		142	8
Oak		60	42	24	24	24 37		19	25	22	20	21 21	9
Weathered													
Sycamore		24	14	24	27	12 20		12	13	13	9	13 12	1
Elm		9	10	9	9	20 11		5	6	9	5	7 6	2
Birch		35	36	32	31	34 34		14	18	14	17	16 16	3
Alder		13	7	18	8	10 11		9	11	13	9	6 9	4
Lime		6	17	14	14	8 12		7	10	9	13	8 9	5
Gean		23	20	22	12	23 20		13	14	13	14	13 13	6
Hazel		14	15	33	9	14 17		14	14	14	12	9 13	7
Oak		8	41	11	19	31 22		15	17	21	13	15 16	8
Pine 25 needles				130-320		213				100-200		141	9

Dunger showed a broad relationship between the preference sequence of litter species to macro-fauna and the water holding capacity of the litter. There were exeptions in that elm *(Ulmus carpinifera)* litter had a high water capacity but a relatively low palatability and that alder and beech litter had widely different palatability but very similar water capacity. The method used by Dunger was (1) to soak the litter for 24 hours in water containing 1% mercuric chloride to prevent microbial decomposition, (2) to dry to constant weight in a saturated atmosphere, (3) to re-weigh after drying at 105°C. This method was used to determine the water holding capacity of some of the litters used in Experiment 5. The results, expressed as percentages of the oven dry weights, are given in Table 5.

Table 5: Palatability of tree litters used in Experiment 5 (1965) in relation to their water holding capacity

Unweathered			Weathered		
	Discs eaten as % of controls	% water capacity		Discs eaten as % of controls	% water capacity
Elm	362	187	Sycamore	386	114
Alder	324	128	Elm	358	153
Sycamore	298	89	Birch	342	65
Birch	281	59	Alder	336	107
Ash	260	98	Lime	331	87
Lime	247	65	Hazel	182	187
Hazel	169	65	Oak	173	64
Gean	143	129	Larch	84	56
Beech	84	68	Pine	74	84
Pine	44	79			
Larch	38	39			
Oak	21	53			

The data suggest that water holding capacity played little part in determining the observed order of preference. In the unweathered litter the more palatable elm and alder had a higher water capacity than the less

palatable beech, oak and conifers but birch was more palatable than gean with less than half its water capacity. Of the weathered litters, hazel had the highest water capacity and an intermediate position in the preference sequence and, although the weathered litters other than elm and alder had higher water capacities than the unweathered litters, there was again little correspondence with the preference order. The values we obtained for water capacity were considerably lower than those reported by Dunger for the same species. There may have been slight differences in the techniques employed but the difference in the results obtained probably reflects real differences between our samples and those of Dunger taken near Leipzig.

CHEMICAL CONSTITUTION

Nitrogen content

Wittich (1953) observed that the litter most readily consumed by earthworms was generally nitrogen rich. In the present study the nitrogen content of the 1963 litters was estimated by the Kjeldahl method.

Table 6: Palatability of tree litters in relation to their nitrogen content

Litter	% of discs taken in preference Experiment	Nitrogen % dry wt.
Elder	45	1.95
Ash	44	1.31
Sycamore	44	1.99
Poplar	42	1.78
Alder	40	2.61
Field Maple	25	1.06
Birch	25	0.84
Walnut	21	0.90
Horse Chestnut	21	0.88
Hazel	21	0.92
Beech	18	0.78
Giant Fir	14	1.02
Pine	11	0.44
Oak	5	0.79

The results agree with those of other workers in that nitrogen rich litters were generally preferred to nitrogen poor litters. Alder might have been expected to have been even more palatable in view of its very high nitrogen content, nevertheless the correlation between palatability and nitrogen content was a strong one.

We took the view that if this relationship were a causative one we should expect some sensory response which would enable the earthworms to detect nitrogenous plant compounds. We therefore carried out preference tests comparing paper discs treated with amino acids with untreated controls. The treatment consisted of dipping the discs into a concentrated solution or, for amino acids difficult to dissolve, coating them with a suspension of the amino acid in water. The results of tests in which 100 treated discs of each kind were put out with the same number of untreated discs in bins of soil containing *L. terrestris* are given below. No difference was found in the response to treated and control discs.

Table 7: Palatability to *Lumbricus terrestris* of paper discs treated with amino acids

Amino Acid	Concentration of solution %	Discs taken	
		Treated	Untreated
DL α Alanine	10	54	52
L Leucine	2.5	62	62
L Glutamic Acid	suspension	76	79
DL Valine	"	75	77
DL Threonine	10	76	80
DL Asparagine	suspension	43	40
DL Serine	"	73	65
L Tyrosine	"	53	52
DL Aspartic Acid	"	25	28
DL Phenyl Alanine	"	46	50
DL Tryptophane	"	70	76
Glycine	20	75	71
DL Iso-Leucine	suspension	61	64
DL Lysine hydrochloride	40	81	82
L Histidine hydrochloride	10	60	66
L Arginine hydrochloride	10	77	72
DL Methionine	suspension	50	64
Amino-n-Butyric acid	30	78	77

Carbohydrate Content

Using electrophysiological methods, Laverack (1960) detected a response to solutions of sucrose and glycerol in prostomial preparations from *L. terrestris*. Mangold (1951) had found earlier that earthworms would feed on gelatine containing sugars. We therefore investigated the soluble carbohydrate content of the litters used in the 1964 preference tests. Hot water extracts gave the following values for soluble carbohydrates determined by the anthrone colorimetric method and expressed as glucose (% dry weight):

Weathered beech 0.7, weathered pine 0.8, pine 0.9, beech 1.1, birch 1.9, oak 2.0, weathered hazel 2.1, hazel 2.5, sycamore 5.5, elder 5.8. A longer series was analysed two months later from the same material which had been stored meanwhile at -10°C.

Table 8: Palatability of tree litters in relation to their soluble carbohydrate content

Litter	% discs taken in 1964 preference experiment		Soluble carbohydrate content % dry wt.	
	Unweathered	Weathered	Unweathered	Weathered
Elder	79	68	4.0	3.4
Alder	68	77	4.5	2.8
Poplar	68	65	3.2	2.6
Sycamore	67	67	4.6	3.4
Ash	60	64	4.0	6.8
Birch	60	67	1.8	2.7
Aspen	58	64	3.5	2.5
Hazel	41	61	2.7	2.1
Oak	38	36	1.5	2.4
Giant Fir	21	23	2.0	1.9
Beech	17	30	0.7	2.4
Pine	12	19	0.7	1.5
Larch	9	9	1.8	1.2

The results show a broad relationship between palatability and carbohydrate content but with individual litters that do not conform to the general pattern. Alder litter, for example, increased in palatability with weathering but decreased in soluble carbohydrate content, and weathered larch which was less palatable than weathered beech contained a considerably higher percentage of soluble carbohydrates.

Preference tests were carried out on paper discs soaked in sugar solutions in the manner described for amino acids. The following sugars were tested: D-glucose, D-mannose, D-galactose, D-xylose, L-arabinose, D-fructose, L-sorbose, sucrose, maltose and raffinose. No response was found to any of the treated discs although some were discernibly sweet to the experimenters. Taking all the tests together, 733 treated discs and 727 untreated controls were taken.

Laverack found that prostomial nerve endings of *L. terrestris* showed a response to 1 M sucrose solution. In view of the negative results given above, further tests were carried out with sucrose solutions using a range of concentrations and carriers. Blotting paper discs soaked in water were tested for palatability as controls against similar discs soaked in sucrose solutions at the following molarities: 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6. In all cases there was no difference in the numbers of control discs and sucrose treated discs taken into the worm burrows. In all the tests combined, 207 control discs and 212 treated discs were taken out of 360 of each given.
Birch, oak and ash litter discs soaked in 10 % sucrose solution were then compared with similar discs soaked in water. Using birch, 23 sucrose treated discs were taken and 24 control discs. Using oak soaked for 65 hours, 15 control discs and 22 sucrose discs were taken. The difference was not significant. Oak discs were left in sucrose solution until it evaporated leaving the discs coated in a syrup. 25 of these were taken and 28 control discs.
The results so far suggested either that earthworms are attracted by substances not yet identified or that they do not have preferences for specific substances such as sugars or nitrogen rich compounds but eat any material which is not distasteful and which is physically acceptable.

Polyphenols

Although there are a number of references in the literature to tannins and other distasteful substances in leaf litter there is little published data. Samples of the litter used in Experiment 1 were extracted with cold 50 % solutions of acetone in water for 12 hours and the total polyphenols were determined by the Folin procedure (Folin & Ciocalteau , 1927) The following values for phenol expressed as % dry weight may be compared with the preference test data in Table 1.

Table 9: Total phenols in the litters used in Experiment 1. (Mean of 2 replicates)

Weathering	Mor				Mull					
	Beech	Oak	Hazel	Birch	Beech	Oak	Hazel	Birch	Ash	Alder
-	5.9	3.9	4.1	3.8	6.5	11.8	1.4	2.7	0.5	4.2
6 weeks	3.5	1.1	0.9	1.1	2.1	2.5	1.1	1.3	0.2	0.9
12 weeks	3.9	0.9	0.4	0.6	1.6	1.3	0.4	0.6	0.2	0.8

The results show a strong correlation with palatability, notably in the rapid loss of polyphenolic constituents during the first 6 weeks of weathering when the palatability of all the litters increased. The difference in polyphenol content of the mor and mull samples of unweathered oak is also strikingly reflected in their palatability. The unweathered alder however, had a high polyphenol content in relation to its palatability.

With the aim of defining the polyphenolic fractions affecting litter palatability more closely, the litters used in Experiment 5 (1965) were analysed for their hydrolysable and condensed tannin contents. The following methods were used:

Hydrolysable tannins (Long, 1961) - 10 g fresh litter were boiled with 200 ml water for ten minutes, filtered, and the filtrate made up again to 200 ml. To 50 ml of the filtrate, 2 ml of 16% ammonium sulphide were added. After standing for 15 minutes the resultant precipitate was filtered off, dried at 105°C and weighed. Condensed tannins (Forsyth, 1955)- 5 g fresh litter were extracted with 100 ml 0.1 N hydrochloric acid, filtered,and the filtrate made up again to 100 ml. To 50 ml of the filtrate, 50 ml of Stiasny's reagent were added. After standing overnight the resultant precipitate was filtered off, dried at 105°C and weighed.

The results show that in the unweathered litters, the order of palatability is strongly associated with the tannin content and that the loss of tannins could account for much of the increased palatability caused by weathering. It fails to account however, for the differences in palatability among the weathered litters, in ten of which no tannins remained. Extracts of the same litter were prepared as previously described for condensed tannin analysis but using 15 g ground litter in 50 ml of 0.1 N HCl to give higher concentrations. These were neutralised to pH 7 with sodium hydroxide solution. The filtrates were absorbed on blotting paper

Table 10: Palatability of tree litters in relation to their tannin content

Litter	Unweathered				Weathered			
	Leaf discs eaten as % of controls	Tannins % dry wt. Condensed	Hydrolysable	Total	Leaf discs eaten as % of controls	Tannins % dry wt. Condensed	Hydrolysable	Total
Elm	362	0	0	0	358	0	0	0
Alder	324	0	0	0	336	0	0	0
Sycamore	298	0	0	0	386	0	0	0
Birch	281	0.22	0.11	0.33	342	0	0	0
Spindle	275	0	0.10	0.10	338	0	0	0
Ash	260	0	1.22	1.22	279	0	0	0
Lime	247	0.40	0.05	0.45	331	0	0	0
Hazel	169	0.20	0.32	0.52	182	0	0	0
Gean	143	0.63	0	0.63	234	0.02	0	0.02
Beech	84	1.96	0.40	2.36	184	0.10	0	0.10
Pine	44	1.81	1.38	3.19	74	0	0	0
Larch	38	1.27	3.95	5.22	84	0	0	0
Oak	21	0.88	0.45	1.33	173	0.05	0	0.05

discs and put out in a preference trial for comparison with leaf litter discs. The numbers of discs taken, expressed as percentages of water-soaked control discs taken are given below and may be compared with the numbers of leaf discs given in Table 10.

Unweathered: Elm 206, Alder 171, Sycamore 183, Birch 154, Spindle 121, Ash 176, Lime 114, Hazel 170, Gean 147, Beech 78, Pine 104, Larch 79, Oak 58;

Weathered: Elm 235, Alder 175, Sycamore 203, Birch 170, Spindle 180, Ash 203, Lime 142, Hazel 168, Gean 206, Beech 185, Pine 168, Larch 154, Oak 115.

It will be seen that the preference order of the leaf discs and the leaf extract discs are broadly in conformity but that this holds both for litters containing tannins and for litters without tannins. There appear therefore to have been some taste substances, other than tannins, in the leaf extract which affect the order of palatability of the litters. What these are is not known.

The palatability of various polyphenolic substances obtained from laboratory suppliers was tested by comparing the numbers of treated blotting paper discs and untreated discs pulled into *L. terrestris* burrows. The treated discs were either coated with a suspension of the substance or dipped into a solution of it; the control discs were dipped in water. In eight tests an average of 45 control discs were taken per test.

Table 11: Palatability of paper discs treated with polyphenols

Polyphenol	Concentration of solution	Treated discs taken as % of control discs taken
Quercetin	suspension	107
Ellagic Acid	suspension	98
Catechol	11%	84
Gallic Acid	suspension	50
D Catechin	suspension	27
Phloroglucinol	10%	26
Protacatechuic Acid	suspension	14
Tannic Acid	5%	0
Tannic Acid on birch discs	5%	0

MICROBIAL ACTION

Dunger (1958) found in his feeding experiments with isopods and diplopods that pedunculate oak leaves increased in palatability after 2 - 3 days soaking and ascribed this to the leaching of tannins. We carried out similar tests with soaked litter fed to *Lumbricus terrestris*. Initially, three tests were made comparing soaked fresh oak litter with unsoaked sycamore litter.

Test	Replicate	Discs taken out of 50: Oak	Sycamore
1. Four lots of 25 oak discs each in 500 ml distilled water shaken for 10 hours then washed	1	15	44
	2	15	43
2. As 1. but shaken for 16 hours with a change of water after 8 hours	1	13	24
	2	13	37

3. Washed in running tap water for $3\frac{1}{2}$ days	1	10	45
	2	9	45

A similar set of tests was made comparing leached and unleached oak discs:

	Replicate	Leached	Unleached
4. Washed in running tap water for 4 days	1	12	12
	2	7	10
5. As 4. but leached for 14 days	1	15	16
	2	26	16

Two final tests were made in which the oak litter was placed outdoors in nets and then incubated in the laboratory with a plentiful supply of water:

	Replicate	Treated	Control
6. Discs outdoors for 48 hours then incubated at 26°C for 14 days	1	42	19
	2	35	5
7. Discs outdoors for 96 hours then incubated at 17°C for 25 days	1	34	12
	2	42	16

After Test 6, sycamore litter discs were soaked in the liquor in which the oak discs has been incubating and their palatability compared with sycamore discs soaked in water. No difference was detected.

From all these tests we concluded that the distasteful substances in oak were probably not leached from the litter during weathering but were broken down by microbial action.

CONCLUSION

The results obtained provide, at the best, a partial and incomplete explanation of why some types of leaf litter are preferred by *Lumbricus terrestris* to others. A variety of factors has been shown to be broadly correlated with palatability including nitrogen content, soluble carbohydrate content and content of polyphenolic substances, especially tannins. Other workers, notably Dunger and Wittich, have shown similar correlations with C/N ratio and calcium content.

But correlation is not causation and the only factor, as yet, for which there is supporting direct evidence of a causative role is that of tannin concentration. Preliminary results suggest that microbial degradation of tannins is important in increasing the palatability of litter during weathering but the difference in acceptability of weathered litters in which there is no tannin remains largely unexplained.

It would be surprising if feeding in earthworms were not, as in other invertebrates, determined in part by phagostimulants. That such phagostimulants exist in leaf litter is suggested by the palatability of paper discs soaked in leaf extracts being greater than discs soaked in water. Our experiments with amino acids and sugars showed no response to any of these substances but many organic litter constituents remain to be tested. Our results suggest that what is important to earthworms in determining the food they select is not yet understood.

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REFERENCES

Dunger, W. (1958). Über die Zersetzung der Laubstreu durch die Bodenmakrofauna im Auenwald. Zool. Jb. (Syst.). 86, 139-180.

Folin, O. and Ciocalteau, V. (1927). On tyrosine and tryptophane determination in proteins. J. Biol. Chem. 73, 627-650.

Forsyth, W. G. C. (1955). Cacao polyphenol substances. 3. Separation and estimation on paper chromatograms. Biochem. J. 60, 108-111.

Laverack, M. S. (1960). Tactile and chemical perception in earthworms. I. Responses to touch, sodium chloride, quinine and sugars. Comp. Biochem. Physiol. 1, 155-163.

Lindquist, B. (1941). Untersuchungen über die Bedeutung einiger skandinavischer Regenwürmer für die Zersetzung der Laubstreu und für die Struktur der Mullerde. Svenska Skogsv. Tidskr. 39 (3), 179-242.

Long, C. (1961). Biochemists Handbook, Spons, London.

Mangold, O. (1951). Experimente zur Analyse des chemischen Sinns des Regenwurms. I. Methode und Verhalten zu Blättern von Pflanzen. Zool. Jahrb. Abt. Allgem. Zool. Physiol. Tiere, 62, 441-512.

Wittich, W. (1953). Untersuchungen über den Verlauf der Streuzersetzung auf einem Boden mit starker Regenwurmtätigkeit. Schriftenr. Forstl. Fak. Göttingen, 9, 1-33

DISCUSSION

L. VLIJM: When an animal has been fed on a special kind of food it may become adapted to this food. Did the earthworms used in your experiment show evidence of such adaptation?

J. E. SATCHELL: I know of no case where such adaptation has been shown for earthworms although I agree one must be careful of such effects in feeding experiments. It would not apply in the present work because different worms were used in the different experiments.

M. S. GHILAROV: The degree of palatability of leaves of various shrubs and trees you have found for *L. terrestris* is of the same character as was found for millipedes by Lyford and D. Sokolov. The latter explained the degree of palatability by the content of calcium. As earthworms are calciphilous animals perhaps the amount of calcium may explain the palatability of leaves for *L. terrestris* too.

J. E. SATCHELL: Calcium content, like nitrogen content, soluble carbohydrate content and polyphenolic content, is one of the factors one expects to find correlated with the palatability of leaf litter. But correlation is not causation and we need direct taste response experiments with calcium compounds before we can say that this factor explains food preferences in any soil animal. The same principle applies to the belief that some earthworms are calciphilous. Calcium content is often correlated with soil pH and although Laverack has demonstrated a direct sensory response by certain earthworms to pH, this has not yet been shown for calcium.

U. THIELE: Did I understand correctly that earthworms refused leaf discs treated with tannic acid? I have been able to show that *Glomeris* feeds on leaf material treated in a 2% tannic acid fluid no less than on untreated material.

J. E. SATCHELL: This surprises me. Our results showed consistently that *L. terrestris* refused to touch litter treated with tannic acid. However tannic acid is not a precisely defined chemical substance and tests on the two animals should perhaps be made with material treated with the same sample of tannic acid.

N. HAARLØV: I should like to ask what you think the earthworms are able to digest. Do they feed on the leaf material, the microflora on the leaves, or their decomposition products?

J. E. SATCHELL: They probably feed to some extent on all three. We know from Barley's work that they digest only about 6% of the insoluble nitrogen that they ingest but, on the other hand, we know that they have the necessary enzymes including cellulase and chitinase to digest some of the plant material they eat.

M. BOUCHÉ: I am very interested by this work which is very important for numerous types of study.

1. What is the position of the blotting paper discs in your classification of the litter discs given in paragraph 2, p. 114 ?
2. I think it is possible that various factors quickly modify the palatability of blotting paper discs. Did you carry out any experiments on this aspect?

J. E. SATCHELL: In answer to 1., the position would be between those litters that were moderately palatable and those that were disliked. In answer to 2. the discs were put out one day and removed the next. We did not find it practicable to give the worms less than one night to make their selection.

P. O. LJUNGSTRÖM: Are there any differences between juvenile and adult *L. terrestris* in their feeding habits and between this and other earthworm species?

J. E. SATCHELL: Juvenile *L. terrestris* are not found feeding on the surface in the same proportion to adults as they occur in the population. This suggests that juveniles feed relatively more on soil organic matter. Of the common peregrine species, *L. terrestris* feeds on the surface more than do others but Lindquist showed that the order of palatability of leaf litters was similar for a range of earthworm species.